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14. ABSTRACT

The typical core temperature (Tc) profile displayed during heat stroke (HS) recovery consists of initial hypothermia followed by delayed hyperthermia. Anecdotal observations led to the conclusion that these Tc responses represent thermoregulatory dysfunction as a result of brain damage. We hypothesized that these Tc responses are mediated by a change in the temperature setpoint. Tc (±0.1°C; radiotelemetry) of male C57BL/6J mice was monitored while housed in a temperature gradient with ambient temperature (Ta) range of 20-39°C to monitor behaviorally selected Ta (Ts) or an indirect calorimeter (Ta=25°C) to monitor metabolism (VO2) and calculate respiratory exchange ratio (RER). Responses to mild and severe HS (thermal area 249.6±18.9 vs. 299.4±19.3°C•min, respectively) were examined through 48h of recovery. An initial hypothermia following mild HS was associated with warm Ts (~32°C), ~35% VO2 decrease and RER ~0.71 that indicated reliance on fatty acid oxidation. After 24h, mild HS mice developed hyperthermia associated with warm Ts (~32°C), ~20% VO2 increase and RER ~0.85. Severe HS mice appeared poikilothermic-like in the temperature gradient with Ts similar to Ts (~20°C) and these mice failed to recover from hypothermia and develop delayed

15. SUBJECT TERMS

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Thermoregulatory, behavioral, and metabolic responses to heatstroke in a conscious mouse model

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Leon LR, Gordon CJ, Helwig BG, Rufolo DM, Blaha MD. Thermoregulatory, behavioral, and metabolic responses to heatstroke in a conscious mouse model. *Am J Physiol Regul Integr Comp Physiol* 299: R241–R248, 2010. First published April 28, 2010; doi:10.1152/ajpregu.00309.2009.—The typical core temperature (T_c) profile displayed during heatstroke (HS) recovery consists of initial hypothermia followed by delayed hyperthermia. Anecdotal observations led to the conclusion that these T_c responses represent thermoregulatory dysfunction as a result of brain damage. We hypothesized that these T_c responses are mediated by a change in the temperature setpoint. T_c (± 0.1°C; radiotelemetry) of male C57BL/6J mice was monitored while they were housed in a temperature gradient with ambient temperature (T_a) range of 20–39°C to monitor behaviorally selected T_a (T_s) or an indirect calorimeter (T_a = 25°C) to monitor metabolism ($\dot{V}O_2$) and calculate respiratory exchange ratio (RER). Responses to mild and severe HS (thermal area 249.6 ± 18.9 vs. 299.4 ± 19.3°C·min, respectively) were examined through 48 h of recovery. An initial hypothermia following mild HS was associated with warm T_s (~32°C), ~35% $\dot{V}O_2$ decrease, and RER ~0.71 that indicated reliance on fatty acid oxidation. After 24 h, mild HS mice developed hyperthermia associated with warm T_s (~32°C), ~20% $\dot{V}O_2$ increase, and RER ~0.85. Severe HS mice appeared poikilothermic-like in the temperature gradient with T_c similar to T_s (~20°C), and these mice failed to recover from hypothermia and develop delayed hyperthermia. Cellular damage (hematoxylin and eosin staining) was undetectable in the hypothalamus or other brain regions in severe HS mice. Overall, decreases and increases in T_c were associated with behavioral and autonomic thermoeffectors that suggest HS elicits anapyrexia and fever, respectively. Taken together, T_c responses of mild and severe HS mice suggest a need for reinterpretation of the mechanisms of thermoregulatory control during recovery.

heat stress; fever; hypothermia; poikilothermia; metabolism

THE CORE TEMPERATURE (T_C) response during recovery from heatstroke (HS) consists of an initial hypothermia followed by delayed hyperthermia (18, 21, 25). In HS patients, hypothermia is thought to be a manifestation of thermoregulatory dysfunction as a result of damage to the central nervous system (CNS) (21). However, there is little evidence to support this hypothesis, as post-mortem analysis of 125 HS patients failed to detect damage to the hypothalamus, considered to be the main site for thermoregulatory control. In experimental animal studies, hypothermia occurs naturally during HS recovery with the depth and duration of this response directly related to intensity of heat strain (14, 25). Hypothermia during HS recovery has been observed in a variety of mammalian species (e.g., guinea pigs, mice, rats, and cats) (1, 18, 25, 29), as well as in an ectotherm (e.g., salamander) (9). Hypothermia may be a critical physiological response for survival and recovery from HS. For example, mice housed at a thermoneutral ambient temperature (T_a; ~30°C) while recovering from HS failed to develop hypothermia compared with mice housed at 25°C; the prevention of this hypothermic response was associated with increased intestinal damage and mortality (18, 29). Hence, it is possible that the hypothermia following recovery from HS is a regulated, adaptive response that is important for survival.

Delayed hyperthermia has been observed in HS patients during the hours, days, and weeks of recovery (21). The occurrence of hyperthermia during the initial hours of recovery was thought to be due to a compensatory peripheral vasoconstriction that occurred following clinical cooling with ice packs on the skin surface. Conversely, protracted hyperthermic episodes were thought to be due to persistent disturbances in thermoregulatory control (21). In experimental animal models, hyperthermia has rarely been observed during HS recovery, presumably because of short-term measurements (typically, <12 h), use of anesthesia, which masked the natural occurrence of this response, or inadequate methods to monitor T_c without stress (1, 25, 29). In a conscious, telemetered mouse model developed in our laboratory, HS induced a biphasic thermoregulatory response consisting of hypothermia during the initial hours of recovery followed by delayed hyperthermia that developed within ~24 h (18). Telemetry is a critical method for the study of T_c responses during HS recovery because long-term measurements can be obtained without the need for anesthesia or other potentially confounding stressors (e.g., handling). Interestingly, the magnitude and duration of the delayed hyperthermia in our mouse model were independent of HS severity and associated with increased circulating levels of the proinflammatory cytokine IL-6 (18, 19). The cytokine and long-term thermal responses observed in conscious HS mice suggests that delayed hyperthermia during recovery may be a regulated T_c response, although this hypothesis has never been tested.

The aim of the current study was to test the hypothesis that HS-induced hypothermia and delayed hyperthermia are regulated T_c responses to a change in the temperature setpoint. To address this aim, behavioral thermoregulation and metabolic responses of telemetered mice were examined in a temperature gradient and indirect calorimeter, respectively, during 48 h of recovery from HS. The use of these techniques was based on the premise that if HS-induced hypothermia and delayed hyperthermia were regulated T_c responses, they would be associated with behavioral and metabolic adjustments that support their development. The contribution of CNS damage to thermoregulatory control during severe HS recovery was assessed in hypothermic mice using hematoxylin-and-eosin staining.

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MATERIALS AND METHODS

Animals. Male C57BL/6J male mice weighing 29.6 ± 0.5 g were individually housed in Nalgene polycarbonate cages (11.5 in × 7.5 in × 5 in) fitted with HEPA-filter cage tops and Alpha-Dri bedding (PharmaServ, Framingham, MA). Rodent laboratory chow (LM-485; Harlan Teklad, Madison, WI) and water were provided ad libitum under standard environmental conditions (25 ± 2°C; 12:12-h light-dark cycle, lights on at 0600). Environmental enrichment consisted of a Nalgene Mouse House (Nalgene Nunc, Rochester, NY) and a maple wood product (Product #W0002; Bio-Serv, Frenchtown, NJ) to encourage climbing and foraging behaviors, respectively. Clean cages and fresh food and water were provided on a weekly schedule. In conducting research using animals, we adhered to the *Guide for the Care and Use of Laboratory Animals* in an Association for Assessment and Accreditation of Laboratory Animal Care-accredited facility. All procedures received Institutional Animal Care and Use Committee approval before experimentation.

Radiotelemetry. Each mouse was intraperitoneally implanted with a battery-operated, free-floating radiotelemetry transmitter device (Model TA10TA-F20; Data Sciences International, St. Paul, MN) for remote sensing of T_c (± 0.1°C). The transmitter weighed ~3.6 g, which represented ~12% of body weight. Body weight was >20 g for all mice on the day of surgery (~2 wk after arrival), which was greater than the manufacturer's recommended nominal body weight for implantation of this transmitter model (www.datasci.com). Although the transmitter was considered free-floating (i.e., it was not sutured to the peritoneal cavity wall), it was unable to freely move in the peritoneal cavity due to its large size and was observed upon examination at the time of death to be residing among the folds of the small intestine. Each transmitter was magnetically activated ~24 h prior to implantation to allow continuous monitoring of T_c during surgical recovery. The radio frequency emitted by each transmitter is directly proportional to T_c; this signal was received by a receiver board antenna under each animal's cage and converted to T_c using predetermined calibration values. Calibration values were verified prior to and following experimentation to ensure accuracy of T_c measurements.

Surgery. Mice were anesthetized with isoflurane anesthesia (2.5% induction, 1% maintenance in 100% O_2), the abdominal fur was shaved and the area scrubbed with a 10% Povidone-iodine solution (Betadine Solution, Purdue Frederick, Stamford, CT) followed by 70% isopropyl alcohol. A ~1-cm incision was made through the skin and abdominal muscle layer using an aseptic technique. Each radiotelemetry device was disinfected by presoaking for 5 h in cold sterilant (Actril; Minntech, Minneapolis, MN) followed by a rinse in 0.9% sterile saline prior to placement in the peritoneal cavity. Rinsate was tested to ensure that no residual Actril was present (<10 ppm). The peritoneal muscle and skin layers were closed with absorbable suture (5–0 Coated Vicryl, RB-1 Taper; Ethicon, Somerville, NJ) using interrupted and continuous subcuticular patterns, respectively. Immediately following surgery, each mouse was placed into a clean cage with ad libitum food and water and returned to the animal room for undisturbed recovery. All mice were provided indomethacin (1 mg/kg) as an analgesic, which was contained on a piña colada-flavored Supreme Mini Treat (Product #F05475–1; Bio-Serv) that was placed into the cage ~1 h prior to surgery and at 0800 h on *days 1*, *2*, and *3* of recovery, as previously described (3); oral consumption of the treat was visually verified daily. Experimentation was not begun until surgical recovery was achieved, as defined a priori as a return to presurgical body weight and food and water intake, as well as stable circadian T_c rhythms (3).

Temperature gradient. Behavioral thermoregulatory responses were determined in a temperature gradient that allowed mice to behaviorally select a range of ambient temperatures during HS recovery (10). Briefly, the gradient runway was constructed of a series of copper bars that were cooled at one end and heated at the other end by circulating cold and hot distilled water through copper tubing that encompassed the exterior ends of the runway. A linear temperature gradient from 18.5 ± 0.2°C to 39.4 ± 0.1°C was maintained along the length of the gradient. The copper runway was surrounded by perforated stainless-steel walls that confined the mouse to the runway, while maintaining adequate air circulation and light penetration for entrainment to the light-dark cycle. Food and water were provided ad libitum at the two ends and middle of the gradient to avoid an influence of food and water availability on the behavioral selection of runway position. Runway temperatures were detected by copper-constantan (type T) thermocouples inserted into the copper bars along the gradient length. The position of the mouse was determined by photocell emitters/detectors at the location of each thermocouple along the runway; runway position is reported as selected temperature (T_s) as determined from the thermocouple readings at 1-min intervals. Three equally spaced radiotelemetry wand receivers (Model RLA-3000, Data Sciences, St. Paul, MN) placed above the stainless-steel walls monitored T_c at 1-min intervals throughout recovery.

Indirect calorimetry. The metabolic responses of mild HS mice only were examined in the indirect calorimeter using the same heat stress protocol (described below) as that used prior to placement in the temperature gradient. Metabolic responses were examined during heat exposure and ~48 h of recovery at T_a of 25°C. This T_a was chosen for direct comparison to earlier studies that characterized HS recovery responses in this species (18). Oxygen consumption ($\dot{V}O_2$; ml/h/kg$^{0.75}$) and carbon dioxide production ($\dot{V}CO_2$; ml/h/kg$^{0.75}$) were measured using a Comprehensive Lab Animal Monitoring System interfaced with Oxymax software (CLAMS; Columbus Instruments, Columbus, OH). Each mouse was housed in a sealed clear Plexiglas cage (permitted entrainment to light-dark cycle; 8 in. × 5 in. × 4 in.) maintained in a temperature-controlled chamber. Note that bedding was not provided in the calorimeter chamber due to potential interference with gas measurements (i.e., bedding traps air). Compressed air of known O_2 and CO_2 concentration was flushed through the calorimeter chamber at a rate of 0.5 l/min (as recommended by the manufacturer), and the expired chamber air was dessicated by drawing it through a canister of Drierite. The dried air was sampled at 1-min intervals by high-speed gas sensing O_2 and CO_2 analyzers (±0.002%). $\dot{V}O_2$ and $\dot{V}CO_2$ values were normalized to body weight$^{0.75}$, although mice of similar body weights were tested in the control and HS condition (28). O_2 and CO_2 analyzers were calibrated prior to each experiment by flushing a calibration gas of known concentration (20.5% O_2, 0.5% CO_2, balance N_2) through the calorimeter chamber to ensure accuracy of O_2 and O_2 measurements. Respiratory exchange ratio (RER) was automatically calculated from $\dot{V}O_2$ and $\dot{V}CO_2$ values, which revealed the energy content of the food stuff utilized by each mouse. Carbohydrate, protein, and fatty acid oxidation yielded RER values of ~1.00, 0.85, and 0.70, respectively (22). T_c values were simultaneously recorded at 1-min intervals by a radiotelemetry receiver board placed under the calorimeter chamber.

Heat stress protocol. The details of the heat stress protocol have been described in detail elsewhere (18). To minimize potential influence(s) of ancillary stressors on T_c and metabolic responses, mice were placed into the heating chambers at normal housing T_a of 25°C for ~24 h prior to experimentation to acclimate to the unique smells and noises of the chambers. Between 0800 and 1000 the following day, T_c was monitored to ensure that mice were in a quiescent, resting state (defined as T_c < 36.0°C, which represents the normal 12-h daytime average T_c for this species; data not shown) prior to heat exposure. Once T_c < 36.0°C was observed, mice were weighed, placed back into the heating chamber in the absence of food and water, and exposed to T_a = 39.5 ± 0.2°C until a preassigned maximum T_c ($T_{c,Max}$) of 42.4°C or 42.7°C was attained. In the temperature gradient experiments, mice were removed from the heating chamber at $T_{c,Max}$, body weight was recorded, and mice were immediately placed onto the copper runway to record T_c and T_s during ~48 h of undisturbed recovery. The direction of placement into the

gradient (facing the cooled or heated end) was randomized between mice to avoid any influence on the behavioral selection of temperatures. For metabolic analysis, mice were only heated to $T_{c,Max}$ = 42.4°C and remained in the calorimeter during all phases of experimentation (i.e., heat exposure and recovery). For both experiments, control mice were exposed to identical conditions at T_a of 25.0°C for the same amount of time as a HS animal to which they were matched; control and HS mice were tested on different days in random order.

Thermoregulatory characteristics. Duration of heat exposure was calculated as the time (min) to reach $T_{c,Max}$ following the initiation of heat stress at *time 0*. Thermal area (°C·min; a measure of thermal load) was calculated as Σ [time intervals (min) × 0.5 (°C above T_c = 39.5°C at the start of the interval + °C above T_c = 39.5°C at the end of the interval)]. Total thermal area was segregated into its ascending (from T_c = 39.5°C to $T_{c,Max}$) and descending (from $T_{c,Max}$ to T_c = 39.5°C) aspects to characterize the heating and cooling aspects of the T_c curves, respectively. Hypothermia was defined as T_c < 34.5°C since this was the lowest T_c value previously observed in male C57BL/6J mice during housing at T_a of 25°C (unpublished observation). Hypothermia depth (°C) was calculated as the lowest 1-h average T_c value, and hypothermia duration (min) was the total time T_c < 34.5°C during recovery. Delayed hyperthermia refers to the T_c elevation observed in mild HS mice that was above that of control mice following hypothermia rewarming, which occurred from 9 to 28 h in the temperature gradient and from 18 to 28 h in the calorimeter.

Survival. Survival was assessed daily by visual inspection and continuous remote sensing of T_c, T_s, and metabolic variables. We hypothesized that access to a range of T_a in the temperature gradient would improve HS recovery, but ~71% of mice (5 out of 7) heated to $T_{c,Max}$ of 42.7°C displayed ~7°C reduction in T_c and were unlikely to survive the 48-h recovery period. To minimize pain and suffering, these mice were killed by CO_2 asphyxiation. On the basis of these results, mice were divided into two groups for post hoc thermoregulatory analysis in the temperature gradient with the "mild HS" group (n = 7, $T_{c,Max}$ 42.4°C and n = 2, $T_{c,Max}$ 42.7°C) representing all animals that survived the 48-h recovery period and the "severe HS" group (n = 5, $T_{c,Max}$ 42.7°C) representing animals that were killed prior to 48 h of recovery. On the basis of these findings, mice were only tested in the mild HS condition (n = 7) in the calorimeter to ensure survival through 48 h of recovery.

Dehydration. All body weight measurements were corrected for transmitter weights. Body weight was determined immediately prior to *time 0* and following $T_{c,Max}$ on a top-loading balance accurate to ± 0.1 g. Dehydration was estimated as [(*time 0* body weight − $T_{c,Max}$ body weight)/*time 0* body weight] × 100.

Tissue injury. Brain histopathology was performed on a different population of mice than those used to examine temperature gradient responses, but ascending thermal area (252.1 ± 17.5°C·min) and dehydration (10.5%) were similar between the mouse populations. Mice were killed at the hypothermic nadir (corresponding to cooling rate of 0.01°C/min) (19) under deep isoflurane anesthesia and perfused with sterile heparinized (10 U/ml) saline prior to removal of the brain. The cerebellum and cerebrum were separated and fixed in 10% neutral buffered formalin (Carson Millonig Formulation, Fisher Scientific, Springfield, MA). Tissues were embedded in paraffin blocks, serially sectioned, and stained with hematoxylin and eosin for microscopic evaluation by a certified veterinary pathologist (IDEXX Veterinary Services, W. Sacramento, CA) at a magnification of ×40 or ×200. Sample size was 8 mice/group. Histopathological analyses of brain tissue from heatstroked animals was compared with time-matched controls to identify characteristic abnormalities based on those previously observed in HS patients and animal models (2, 4, 21).

Statistical analysis. Data are presented as means ± SE. T_c, T_s, $\dot{V}O_2$, and RER were analyzed and presented as 1-h averages for ease of presentation, unless otherwise indicated. Two-way ANOVA with Holm-Sidak post hoc tests (SigmaStat 3.5; Systat Software, Chicago, IL) determined main group and time effects for $T_{c,Max}$, total, ascending and descending thermal area, dehydration, hypothermia depth, hypothermia duration, and fever. Significance was set at $P < 0.05$.

RESULTS

Heat stress profiles. Prior to heat exposure, all mice showed normal circadian T_c patterns that consisted of low daytime (inactive period; ~36°C) and high nighttime (active period; ~38°C) values that are characteristic of this species (17). Prior to heat exposure, body weight of control, mild, and severe HS mice were virtually identical (~30 g, Table 1). Post hoc analysis of heat stress profiles showed a trend toward higher heat exposure times (i.e., time to reach $T_{c,Max}$), total, ascending (heating), and descending (cooling) thermal areas in severe HS mice, but these values were not significantly different from those observed in mild HS mice (Table 1). Heat stress induced ~8–10% dehydration, which was similar between mice in the mild and severe HS groups and significantly greater than that

Table 1. *Characteristics of mice during heat exposure and recovery in a temperature gradient*

	Control (n = 8)	Mild HS (n = 9)	Severe HS (n = 5)	P Value
Heat exposure				
Starting body weight, g	29.8 ± 0.8	30.2 ± 0.6	30.2 ± 0.6	0.91
Time to $T_{c,Max}$, min		243 ± 24	277 ± 21	0.35
Total thermal area, °C·min		249.6 ± 18.9	299.4 ± 19.3	0.12
Ascending thermal area, °C·min		234.9 ± 18.6	273.9 ± 23.0	0.22
Descending thermal area, °C·Min		14.7 ± 2.7	25.4 ± 10.2	0.22
Dehydration, %	2.0 ± 0.4[a,b]	8.5 ± 1.0[a]	10.3 ± 0.8[b]	<0.001
Recovery				
Hypothermia depth, °C		34.0 ± 0.3	19.7 ± 0.5	<0.001
Hypothermia duration, min		107 ± 16	775 ± 163	<0.001
Hypothermia T_s, °C	29.5 ± 0.8[a,b]	32.1 ± 0.8[a,c]	19.7 ± 0.5[b,c]	<0.001
Delayed hyperthermia, °C	36.2 ± 0.2	37.0 ± 0.2		0.012
Delayed hyperthermia T_s, °C	32.6 ± 0.7	31.4 ± 0.4		0.20

Data are expressed as means ± SE. Heat exposure occurred in a chamber that was separate from the temperature gradient. $T_{c,Max}$, maximum core temperature. T_s, behaviorally selected temperature in a temperature gradient. Sample sizes (n) are indicated in parentheses. [a,b,c]Values with similar letter designations represent significant differences between groups, with significance set at $P < 0.05$. Mild heatstroke (HS) denotes mice that survived 48-h recovery in the temperature gradient. Severe HS denotes mice heated to $T_{c,Max}$ = 42.7°C that were not expected to survive 48 h in the temperature gradient (~71%). Hypothermia depth represents the lowest 1-h average T_c value observed during recovery; hypothermia T_s represents temperatures behaviorally selected by mice in the temperature gradient during hypothermia. Delayed hyperthermia represents the average T_c value displayed in each group from 9 to 28 h of recovery; delayed hyperthermia T_s represents the average T_s behaviorally selected by mice in the temperature gradient when delayed hyperthermia was observed.

observed in control mice (Table 1). The ~2% dehydration incurred by control mice was presumably due to the absence of food and water during experimentation.

Behavioral responses of mild HS mice in a temperature gradient. Control mice showed an initial hyperthermia upon placement in the gradient (*time 0)* that peaked at 38.4 ± 0.3°C and did not subside to a normal baseline level of 36.1 ± 0.3°C until ~3 h (Fig. 1*A*). This increase in T_c correlated with the behavioral selection of relatively low temperatures in the gradient (T_s = 29.9 ± 0.8°C) compared with those normally selected by control mice during quiescent periods of the lights-on phase, such as that observed on the second day of recovery when the mice were not handled (i.e., 22–28 h, T_s = 32.4 ± 0.7°C; Fig. 1*B*). From ~4 h through the remainder of experimentation, T_c and T_s tended to be inversely correlated with one another, as mice behaviorally selected warm temperatures (T_s ~32–34°C) when T_c was low during the lights-on period, and cooler temperatures (T_s ~28–32°C) during the lights-off period when T_c was elevated (Fig. 1).

Immediately following placement in the temperature gradient, mild HS mice cooled at a rate of 0.1°C/min until hypothermic depth of 34.0 ± 0.3°C was reached at 1 h, which represented a significantly lower T_c than that observed in control mice (Fig. 1*A*; ANOVA, $P < 0.05$). Note that mild HS mice behaviorally selected higher temperatures than control mice during hypothermia development (32.1 ± 0.8°C vs. 29.5 ± 0.8°C; Fig. 1*B* and Table 1, ANOVA, $P < 0.05$). Starting at ~3 h after placement in the gradient, mild HS mice showed a rapid (~107 min) rewarming from hypothermia through the first night, and T_s remained at ~32°C during this period; T_c of mild HS mice peaked at 37.9 ± 0.3°C at ~10 h and remained elevated above controls most of the following day (9–28 h, T_c = 37.0 ± 0.2°C vs. 36.2 ± 0.2°C; Table 1 and Fig. 1, ANOVA, $P = 0.012$). This delayed hyperthermia was associated with the selection of similar T_s in control and mild HS mice during this period (T_s ~31–33°C, Table 1 and Fig. 1; ANOVA, $P = 0.20$). With the exception of the transitions between the inactive and active periods (i.e., 19–21 h, and 31–33 h), T_c and T_s values were similar between control and mild HS mice during the lights-off periods (Fig. 1).

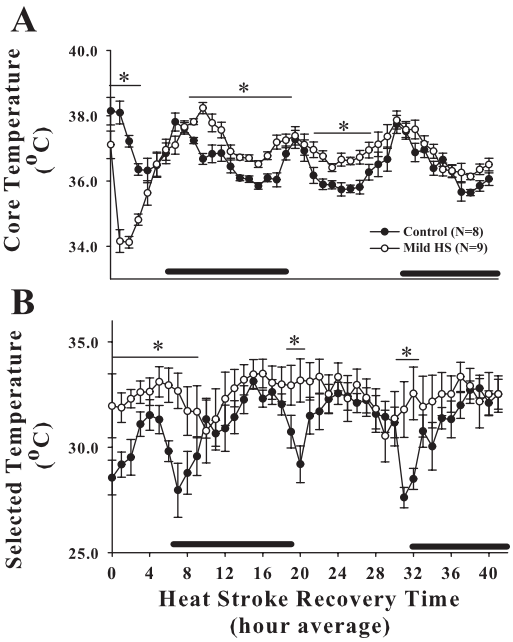

Fig. 1. Core temperature (T_c; *A*) and selected temperature (T_s; *B*) of control and mild heatstroke (HS) mice during recovery in a temperature gradient. *Time 0* refers to the start of recovery after mice were removed from the heat stress environment and placed into the temperature gradient. Data are 1-h averages. Solid horizontal bars indicate lights-off periods. *Significant difference between HS and control animals at $P < 0.05$.

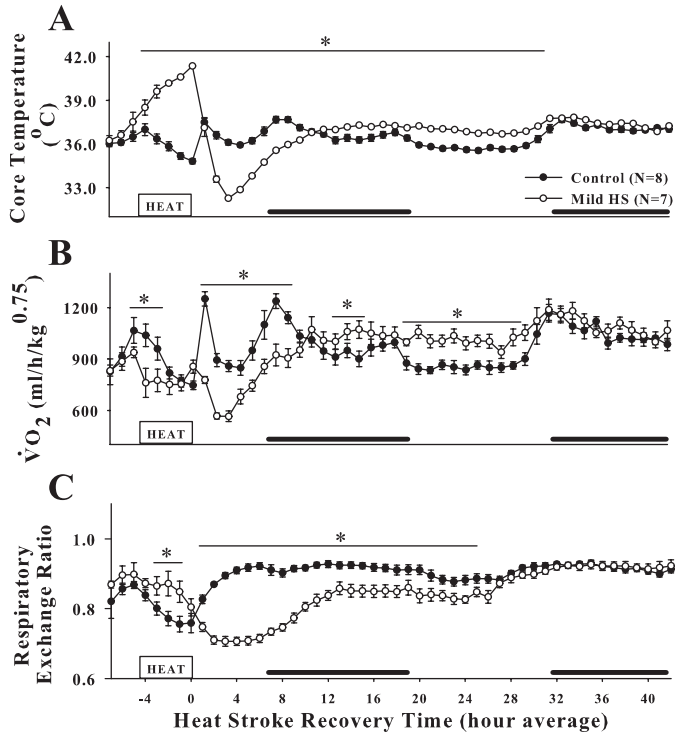

Fig. 2. T_c (*A*), oxygen consumption ($\dot{V}O_2$; *B*), and respiratory exchange ratio (RER; *C*) of control and mild HS mice during heat exposure and recovery in an indirect calorimeter. *Time 0* represents the start of recovery in the indirect calorimeter at ambient temperature (T_a) of 25°C. Data are 1-h averages. Black horizontal bars indicate lights-off periods. *Significant difference between HS and control animals at $P < 0.05$.

Metabolic responses of mild HS mice. The thermoregulatory profiles displayed by control and mild HS mice during heat exposure and recovery in the calorimeter are presented in Fig. 2*A*. Handling and weighing the control mice were the apparent cause of a transient (~120 min) increase in T_c that was observed immediately prior to heat exposure and at 1 h (Fig. 2*A*). Other than these perturbations, control mice displayed a normal circadian T_c rhythm for this species, which consisted of low daytime (~36°C) and high nighttime (~38°C) values (Fig. 2*A*). Mild HS mice in the calorimeter required a longer time to reach $T_{c,Max}$ (335 ± 23 min), accrued a greater total (407.6 ± 27.3°C·min) and ascending thermal area (392.1 ± 27.4°C·min), but descending area (15.5 ± 1.9°C·min) and dehydration (~11%) were similar to mild HS mice heated in a separate chamber in the temperature gradient experiment (compare Table 1 vs. Table 2; $P < 0.001$). These differences were presumably due to the high air flow rate (0.5 l/min) required in the calorimeter chamber, which likely facilitated heat dissipation during heat exposure. A cooling rate of ~0.1°C/min in the calorimeter was similar to that observed in the temperature gradient, but the hypothermic depth (32.3 ± 0.1°C) and duration (341 ± 46 min) was more pronounced (compare Tables 1 and 2; ANOVA, $P < 0.001$). Mice rewarmed from

Table 2. *Characteristics of mice during heat exposure and recovery in a calorimeter*

	Control (n = 10)	Mild HS (n = 9)	P Value
Heat exposure			
Starting body weight, g	29.1 ± 0.4	28.6 ± 0.3	0.31
Time to $T_{c,Max}$, min		335 ± 23	
Total thermal area, °C·min		407.6 ± 27.3	
Ascending thermal area, °C·min		392.1 ± 27.4	
Descending thermal area, °C·min		15.5 ± 1.9	
Dehydration, %	3.8 ± 0.5	10.6 ± 0.9	0.04
Recovery			
Hypothermia depth, °C	36.1 ± 0.1	32.3 ± 0.1	<0.001
Hypothermia duration, min		341 ± 46	
Delayed hyperthermia, °C	35.8 ± 0.0	37.0 ± 0.1	<0.001

Data are expressed as means ± SE. Heat exposure and recovery characteristics were determined in a calorimeter. $T_{c,Max}$, maximum core temperature. Sample sizes (n) are indicated in parentheses. Significance, set at $P < 0.05$. Hypothermia depth represents the lowest 1-h average T_c value observed during recovery. Delayed hyperthermia represents the T_c value displayed in each group from 18-28 h of recovery Note that mice were only heat stressed to $T_{c,Max}$ of 42.4°C.

hypothermia and displayed hyperthermia compared with control mice from 18–28 h (37.0 ± 0.1 vs. 35.8 ± 0.0°C, respectively; Table 2 and Fig. 2*A*; $P < 0.001$). By the second night, T_c profiles did not differ between control and mild HS mice in the calorimeter.

On the day of experimentation, resting $\dot{V}O_2$ was similar between groups until the initial body weight measurement, which induced ~30% increase in $\dot{V}O_2$ of control mice (Fig. 2*B*). Control mice showed a second weighing-induced increase in $\dot{V}O_2$ at ~1 h that corresponded to the time of the second weighing and return of food and water to the cage; control mice then displayed a circadian $\dot{V}O_2$ profile that mirrored the T_c rhythms with high nighttime and low daytime values (Fig. 2*B*). $\dot{V}O_2$ of mild HS mice was reduced below control levels during heat exposure and then showed an abrupt ~35% decrease at 2 h, which preceded the attainment of hypothermia depth (Fig. 2, *A* and *B*). Starting at 4 h, mild HS mice showed a progressive increase in $\dot{V}O_2$, which peaked at ~10 h and remained ~20% elevated above controls during hyperthermia (ANOVA, $P < 0.05$; Fig. 2, *A* and *B*). During the second night, $\dot{V}O_2$ of control and mild HS mice were virtually indistinguishable (Fig. 2*B*).

Prior to heat exposure, RER of control and mild HS mice was ~0.88 (Fig. 2*C*). Control mice showed a gradual decline in RER during the period in which food and water were absent from the cage and reached a nadir of ~0.76 at 0 h; the subsequent rebound of control mouse RER toward baseline levels started at the time that food was returned to the cage (0 h) and remained at ~0.90 until the end of experimentation (Fig. 2*C*). RER of mild HS mice reached a nadir of ~0.71 by 2 h and remained significantly depressed below control levels until the second night (Fig. 2C, $P < 0.001$). From 27 h to the end of experimentation, control and mild HS mice showed similar RER values.

Behavioral responses of severe HS mice in a temperature gradient. The control group depicted in Fig. 3 represents the same mice as those shown in Fig. 1, and the details of their T_c and T_s recovery responses are described above. Upon placement in the temperature gradient, severe HS mice cooled at a rate of 0.8°C/min, became severely hypothermic with T_c of 19.7 ± 0.5°C at 2 h (Fig. 3*A* and Table 1). This T_c response was associated with the behavioral selection of ~20°C in the temperature gradient (Fig. 3*B* and Table 1), such that severe HS mice appeared poikilothermic-like in the gradient. (Fig. 3 shows these T_c and T_s responses through 10 h of recovery only, as this response was maintained throughout recovery.) Two severe HS mice were physically moved by an investigator from the cool end of the gradient to a warmer location of ~30°C to determine whether these animals maintained locomotor capabilities following this level of heat severity. Although physical movement to a warmer location rapidly increased T_c, each mouse eventually returned to the cool end of the gradient (T_s ~19.5°C) by its own volition with T_c and T_s at similar levels within a few hours (Fig. 4).

Tissue histopathology. Representative photomicrographs showing transverse sections of the brain of a control (*A*, *C*, *E*) and HS (*B*, *D*, *F*) mouse are shown in Fig. 5. Neuronal morphology and density appeared normal in the hypothalamus (HY; top, ×40 magnification; middle, ×200 magnification) in control (Fig. 5, *A* and *C*) and HS mice (Fig. 5, *B* and *D*). In the cerebellum, the outer molecular (M) and inner granular (G) layer consisted of small neurons whose morphology and density appeared similar between control and HS mice (Fig. 5, *E* and *F*). The Purkinje cells occurred at the interface of the molecular and granular layers, with normal appearance in the control and HS mice (black arrows in Fig. 5, *E* and *F*).

DISCUSSION

There are several reports documenting hypothermia and delayed hyperthermia during HS recovery in patients and experimental animal models (1, 11, 18, 21, 25, 29). Although these anecdotal observations have led to the conclusion that these T_c responses represent thermoregulatory dysfunction as a result of CNS damage (21), the thermoeffector mechanisms

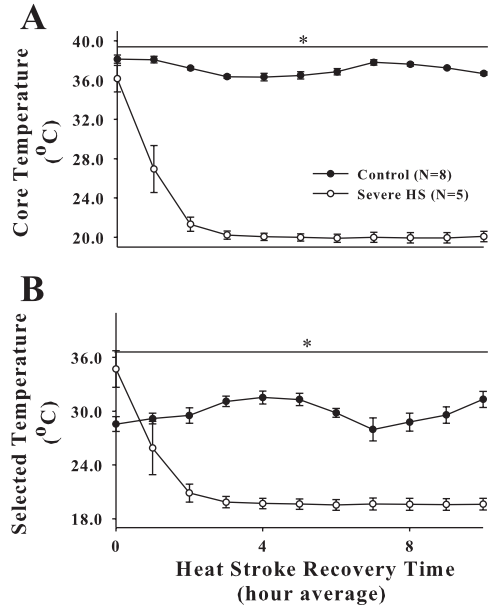

Fig. 3. T_c (*A*) and T_s (*B*) of control and severe HS mice during recovery in a temperature gradient. *Time 0* refers to the start of recovery after mice were removed from the heat stress environment and placed into the temperature gradient. Data are 1-h averages. *Significant difference between HS and control animals at $P < 0.05$.

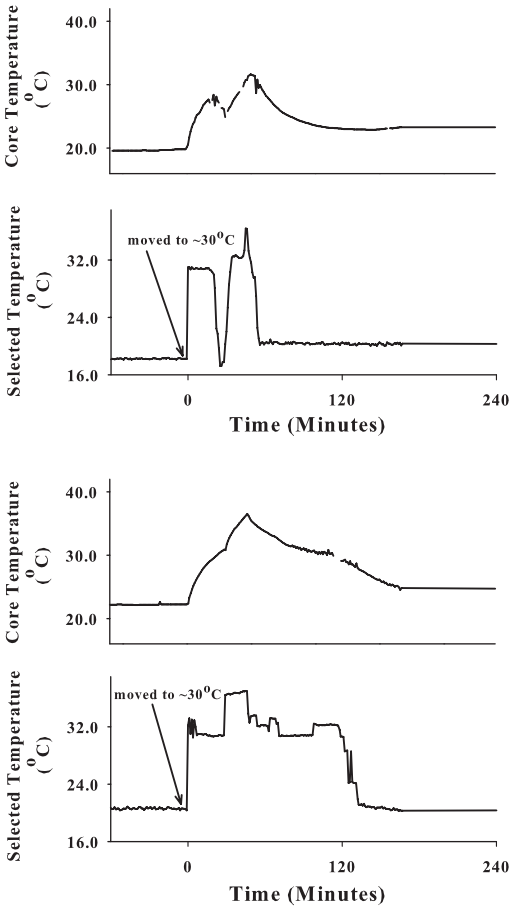

Fig. 4. T_c and T_s responses of two mice that were physically moved from the cold end (~20°C) of the temperature gradient to a warmer locale (~30°C) to assess locomotor function during severe HS recovery. *Time 0* represents the time that each mouse was physically moved by an investigator to the warmer temperature gradient location. Data are given in 1-min intervals.

mediating these responses have never been examined in detail. The present study showed that the T_c responses and mechanisms of thermoregulatory control used by mice during HS recovery differed depending on the level of HS severity. During recovery from mild HS, mice exhibited an initial decrease followed by a delayed increase in T_c when housed in an indirect calorimeter maintained at T_a of 25°C, as well as in a temperature gradient with a range of T_as that permitted the mice to behaviorally control T_c. These HS-induced T_c responses appeared to be predominantly regulated by metabolic adjustments, as they were associated with significant changes in metabolic rate (~35% decrease and ~20% increase in $\dot{V}O_2$, respectively) while the T_a selected in the temperature gradient was slightly elevated or similar to that observed in control mice (~31–32°C). We contend that these data support the hypothesis that the decrease and increase in T_c observed during recovery from mild HS are regulated processes, as a result of a change in the temperature setpoint. In other words, mild HS elicits anapyrexia and fever, respectively. Conversely, severe HS mice developed anapyrexia but appeared poikilothermic-like in the temperature gradient with T_c similar to T_s and did not recover and develop fever. Despite the absence of cellular damage to the hypothalamus and other CNS regions, severe HS mice appeared to experience dysfunction in thermoregulatory control as corrective behavioral adjustments were not invoked to recover from anapyrexia and prevent sustainment of the profound reduction in T_c (~20°C) that would most likely have been lethal.

Behavioral and metabolic responses in mild HS mice. Hypothermia has been reported in rodents during recovery from HS (18, 29), but the regulated nature of this T_c response has never been determined in mammals. In the temperature gradient, mild HS mice selected a T_a range that was slightly warmer compared with nonheated controls during the initial hours following HS collapse. Although regulated reductions in T_c are often characterized by preference for relatively cool T_a (9), the behavioral responses observed in the current study in response to mild HS do not necessarily contradict our hypothesis that this was a regulated event. Mild HS mice selected a T_a range that supported a reduction in T_c, but at a depth and duration that was attenuated (T_c ~ 34°C, ~107 min) compared with that observed when housed at a constant T_a of 25°C in the calorimeter (T_c ~ 31–32°C, ~341 min). The marked (~35%) reduction in metabolic thermogenesis following mild HS in the calorimeter leads one to expect that mice housed in the gradient had a similar reduction in metabolic heat production, meaning that selection of temperatures cooler than 31–32°C would have led to a deeper and more prolonged reduction in T_c. We interpret these data to mean that access to behavioral mechanisms of thermoregulatory control in the temperature gradient allowed mild HS mice to more

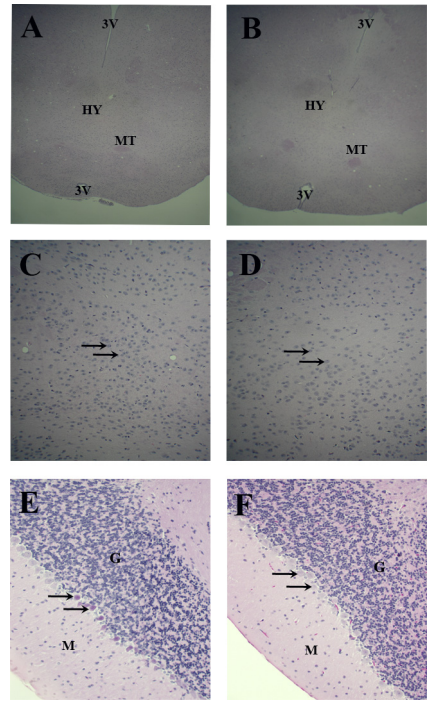

Fig. 5. Representative photomicrographs from C57BL/6J mice exposed to the control (*A*, *C*, *E*) or severe HS condition (*B*, *D*, *F*). Tissues were collected at hypothermia depth and stained with hematoxylin and eosin for microscopic evaluation. Top (×40): transverse sections showing normal neuronal morphology of a control (*A*) and severe HS mouse brain (*B*). Hypothalamus (HY); third ventricle (3V); mammillothalamic tract (MT). Middle (×200): transverse section of the hypothalamus showing normal neuronal morphology of a control (*C*) and severe HS mouse (*D*). *C* and *D*: nuclei are identified by black arrows. *E* and *F*: (×200): transverse section through the cerebellum showing Purkinje cells (black arrows) at the interface of the molecular (M) and granular (G) layers with normal appearance in a control (*E*) and severe HS (*F*) mouse.

precisely regulate T_c responses during recovery. It is also important to note that mild HS mice selected a T_a of ~31–32°C despite having access to T_as as high as 39°C. Selecting the warmest part of the gradient would have clearly prevented the reduction in T_c following mild HS. This indicates that behavioral thermoregulation in mild HS is operative, and mice attempt to regulate T_c around 34°C.

Several autonomic mechanisms of thermoregulatory control have been hypothesized to mediate HS-induced reductions in T_c, including, thermoregulatory instability (21), reversible metabolic inhibition (25), and widening of the interthreshold zone (i.e., the temperature range for activation of thermoeffector responses; 13, 25). In the calorimeter, mild HS mice showed ~35% decrease in $\dot{V}O_2$, which preceded the nadir of the T_c decrease. Anapyrexia is defined as a decrease in T_c due to a regulated change in the setpoint that is actively established and defended by heat-loss thermoeffector mechanisms (13). Hence, the association of reversible metabolic inhibition with the reduction in T_c following HS collapse suggests that this was a response to a regulated reduction in the setpoint. Anapyrexia is a mechanism commonly used by small rodents to survive exposure to environmental extremes, such as dehydration, hypoglycemia, and infection and its protective effects are thought to reside in the minimization of metabolic demands during conditions of severe energy depletion, tissue injury, or infection (5, 12, 14). It is important to note that dehydration, hypoglycemia, and tissue injury are typical responses observed in our mouse HS model (18, 19). Mice and other small mammals are metabolic specialists, meaning they rely primarily on metabolic adjustments to control body temperature (23). Although metabolism was not monitored in mice housed in the temperature gradient, it is reasonable to assume that mild HS mice used reversible metabolic inhibition as a mechanism of T_c control in this environment, as well. This assumption is based on the observation that mice displayed ~2°C T_c reduction in the temperature gradient during the selection of ambient temperatures ~7°C warmer than that provided in the calorimeter.

Delayed hyperthermia has been clinically documented in patients during the hours and days of HS recovery but has typically not been reported in animal HS models due to the use of experimental techniques (e.g., restraint, anesthesia) that prevented long-term studies (1, 21, 25, 29). Although delayed hyperthermia is clinically reported as fever in HS patients, the regulated nature of this response has apparently never been examined. The use of radiotelemetry in conscious mice allowed us to examine the regulated nature of this delayed T_c increase in mild HS mice. Following recovery from anapyrexia, the T_c of mild HS mice was ~0.8°C higher than that displayed by nonheated controls despite selection of a similar T_a range in the temperature gradient. This behavioral response is similar to those observed in an experimental model of bacterial infection in which rats were systemically injected with LPS (a cell wall component of gram-negative bacteria) and allowed to recover in a temperature gradient (8, 26). Despite the selection of similar T_s in the gradient, LPS-injected rats developed fever compared with saline-injected controls and showed a significant increase in metabolic heat production in a calorimeter (8, 26). In mild HS mice, the delayed increase in T_c was associated with ~20% increase in $\dot{V}O_2$ in the calorimeter. Fever is defined as a regulated increase in the setpoint that is actively established and defended by the operation of heat-conserving and heat-producing thermoeffectors (13). Hence, selection of relatively warm T_s combined with increased metabolic heat production constitute heat-conserving and heat-producing mechanisms to raise T_c and support our hypothesis that the delayed T_c increase in mild HS mice is a regulated response akin to fever. Given the high metabolic (energetic) cost of developing and sustaining fever, the fact that it was observed in mild HS mice during reliance on fatty acid oxidation speaks to the importance of this response for HS recovery. While fever's adaptive value during infection is recognized (15), its impact on HS recovery is unknown. Future experiments will need to examine the effect(s) of fever-reducing agents, such as nonsteroidal anti-inflammatory drugs (NSAIDs; e.g., aspirin, ibuprofen) on HS outcome to further delineate the importance of this T_c response for recovery. Although aspirin was effective in reducing T_c of dehydrated rats during heat exposure, NSAIDs have never been tested for their effects on the fever response displayed during recovery (27).

Behavioral responses of severe HS mice in a temperature gradient. Severe HS mice exhibited cold-seeking behavior in the temperature gradient and appeared poikilothermic-like as T_c was nearly equal to T_s. Poikilothermia is typically observed as large variations in T_c (as a function of T_a) in organisms without effective autonomic temperature regulation (13). Although it has been suggested that this mechanism of T_c control is protective for rodents during conditions of energy depletion (24), poikilothermia is typically regarded as a clinical sign of thermoregulatory failure at the level of the hypothalamus (20). For example, poikilothermia is observed following exposure to anesthesia and other drugs, which are known to affect thermosensitive neurons of this brain region (7). Interestingly, a poikilothermic patient required 10–20 times greater than normal reductions in T_c to trigger autonomic thermoregulatory defenses, whereas behavioral thermoeffector responses and cognitive function remained intact (16). Unfortunately, the low survival rate of severe HS mice precluded us from measuring metabolic (autonomic) responses in this study. However, results from our intervention study suggest that a decrease in the setpoint stimulated cold-seeking behavior in severe HS mice for development of anapyrexia. That is, when mice were physically moved by an investigator from 19°C to 30°C, they eventually resumed cold-seeking behavior and decreased T_c to 20°C. In healthy animals, peripheral thermal receptors are stimulated following a reduction in T_c and transmit afferent information to the hypothalamus for integration and stimulation of corrective actions to maintain tight thermoregulatory control and prevent potentially life-threatening reductions in T_c. The absence of these tightly controlled corrective actions appeared to inhibit recovery from anapyrexia in severe HS, suggesting that thermoregulatory control mechanisms were disrupted in these animals. Although we were unable to detect cellular damage to the cerebellum, hypothalamus, or other brain regions, we cannot rule out the possibility that cold-seeking behavior was not due to a widening of the interthreshold zone, desensitization of peripheral thermal receptors, or dysfunction at the CNS level, such that afferent thermal receptor signals were not properly integrated. Taken together, our data suggest that recovery from HS involves activation of cold-seeking mechanisms, but in the transition from mild to severe forms of this disorder, the regulation of this behavior

becomes pathological, such that morbidity and mortality may be exacerbated.

Perspectives and Significance

Environmental heat exposure is one of the deadliest natural hazards in the United States with ~200 deaths per year (6). If the current upward trend in heat wave incidence and climate change toward global warming continues, we may expect HS mortality rates to continue to increase over the ensuing decades unless medical advances in clinical treatments are soon realized. An understanding of the regulated nature of heat-induced T_c responses is instrumental for the development of more effective HS treatments to minimize morbidity/mortality. While the T_c responses observed during HS recovery are traditionally regarded as clinical signs of thermoregulatory failure in HS patients, it is intriguing to speculate that these endogenous mechanisms are protective in promoting recovery in this syndrome. Clearly, our understanding of the physiological mechanisms of T_c regulation during HS recovery requires further study to determine their impact on morbidity and long-term outcome.

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